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A Mutation in the Glut2 Glucose Transporter Gene of a Diabetic Patient Abolishes Transport Activity*

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Glut2, the facilitative glucose transporter isoform expressed in pancreatic β cells, is believed to play a role in glucose-stimulated insulin secretion. Two polymorphisms that result in amino acid substitutions have been reported in the human Glut2 gene (Tanizawa, Y., Riggs, A. C., Chiu, K. C., Janssen, R. C., Bell, D. S. H., Go, R. P. C., Roseman, J. M., Acton, R. T., and Permutt, M. A. (1994) *Diabetologia* 37, 420–427). A threonine 110 → isoleucine substitution was present at equal frequency in diabetic and control populations, and a valine 197 → isoleucine substitution was discovered in a single allele of a patient with non-insulin-dependent diabetes. The effect of these amino acid changes on glucose transport activity was tested by expression of the mutant proteins in *Xenopus* oocytes. The polymorphism at threonine 110 had no effect on the expression of Glut2 protein or the uptake of 2-deoxyglucose. Remarkably, however, the highly conservative valine 197 → isoleucine amino acid change abolished transport activity of the Glut2 transporter expressed in *Xenopus* oocytes. This represents the first known dysfunctional mutation in a human facilitative glucose transporter protein. The presence of this mutation in a diabetic patient suggests that defects in Glut2 expression may be causally involved in the pathogenesis of non-insulin-dependent diabetes.

Glut2 is a high K_m facilitative glucose transporter expressed in liver, pancreatic β cells, kidney, and intestine (1–4). This glucose transporter isoform is distinguished by its relatively low affinity for glucose (5). Coupled with glucokinase, the high K_m form of hexokinase, Glut2 has been proposed to act as part of the glucose sensing mechanism responsible for glucose-stimulated insulin secretion in pancreatic β cells (6). If this hypothesis is correct, then reductions in β cell Glut2 might give rise to the reduced insulin secretion associated with non-insulin-dependent diabetes (7). Reductions in β cell Glut2 have been observed in several animal models of diabetes, including the autoimmune diabetic BB rat (8), the Zucker diabetic fatty rat (9, 10), the obese, diabetic, db/db mouse (11), and the streptozotocin-treated rat (12). The reductions in Glut2 in these models is associated with a loss in glucose-stimulated insulin secretion. However, subsequent experiments argue against a reduction in Glut2 being the cause of reduced β cell glucose sensitivity in some of these rodent models. Experiments in which islets from diabetic db/db mice and normal db/+ mice were cross-transplanted under kidney capsules indicated that the loss of β cell Glut2 was a consequence of the diabetic state rather than a preceding factor. Glucose stimulated insulin release could be restored in perfused pancreas from streptozotocin-treated rats by perfusion in buffer lacking glucose, with no increase in β cell Glut2 (13). Transgenic mice overexpressing a Ras oncoprotein in β cells also exhibit a dramatic decrease in β cell Glut2 expression (14). However, these mice are euglycemic, and their β cells exhibit normal glucose-sensitive insulin secretion. Isolated islets from these transgenic mice displayed normal glucose phosphorylation and oxidation, consistent with the non-rate-limiting role of Glut2 in β cell glucose metabolism. Thus, the role of Glut2 in these animal models of diabetes remains unclear.

Two polymorphisms have recently been described in the human Glut2 gene that result in amino acid substitutions (15). One polymorphism is equally prevalent in control subjects and patients with non-insulin-dependent diabetes (NIDDM)[1] and changes threonine 110 to an isoleucine residue. The presence of a threonine at position 110 is unique to the Glut2 isoform, as all of the other human Glut isoforms normally have an isoleucine residue at this position. The second polymorphism changes valine 197 to an isoleucine residue and was detected in only one of 48 female African-American patients with gestational diabetes whose diabetes resolved after pregnancy. This mutation was not present in any of the 52 control subjects examined. Valine 197 is conserved in Glut1 through Glut4. We report here that the valine 197 → isoleucine mutation abolishes transport activity of Glut2 expressed in *Xenopus* oocytes. This mutation may therefore contribute to the development or pathogenesis of diabetes in this patient.

EXPERIMENTAL PROCEDURES

The procedures for preparation and injection of *Xenopus* oocytes, mRNA synthesis, isolation of oocyte membranes, and 2-deoxyglucose uptake assays have been described in detail previously (16–18).

Construction of Glut2 Mutants—Human Glut2 cDNA was a kind gift from Drs. Chuck Burant and Graeme Bell, University of Chicago School of Medicine. Glut2 and Glut1 (19) cDNAs were subjected to site-directed mutagenesis using the Clontech Transformer kit (Clontech Laboratories, Palo Alto, CA). The G → A mutation was introduced into codon 197 of the human Glut2 cDNA using the oligonucleotide 5′-GCTGGCCATCATCACGGGCAT-3′, and the resulting mutant cDNA was reverted back to wild-type using the oligonucleotide 5′-CTGGCCATCGTCACGGGCATT-3′. The corresponding mutation was introduced into codon 165 of human Glut1 cDNA using the oligonucleotide 5′-CTGGGCATCATCGTCGGCATC-3′. The threonine → isoleucine change at codon 110 of Glut2 was introduced into the cDNA using the oligonucleotide 5′-GGTGGAATGATTGCATCATTC-3′. The mutant cDNAs were sequenced completely to verify the presence of the desired mutation. *In vitro* synthesis of capped mRNAs was performed using the Megascript kit (Ambion, Austin, TX).

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[1] The abbreviation used is: NIDDM, non-insulin-dependent diabetes.

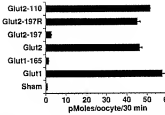

FIG. 1. **2-Deoxyglucose uptake activity of glucose transporter mutants.** Stage 5 *Xenopus* oocytes were injected with either water (*Sham*) or 50 ng of the indicated wild-type or mutant glucose transporter mRNA. Three days after injection uptake of [^{3}H]2-deoxyglucose (25 μM) was performed on groups of 15 oocytes as described previously (11). Values represent mean ± S.E. The absence of an error bar indicates the error was too small to be shown graphically. *Glut2 110*, Glut2 with isoleucine substituted for threonine at position 110; *Glut2-197R*, a Glut2 clone in which the valine 197 → isoleucine mutation was reverted back to valine; *Glut2-197*, Glut2 with isoleucine substituted for valine at position 197; *Glut1-165*, Glut1 with isoleucine substituted for valine at position 165.

Detection of Glucose Transporter Proteins by Laser Confocal Immunofluorescence Microscopy and Western Blotting—Three days after injection, oocytes either were used for membrane preparation or were frozen, sectioned, and subjected to indirect immunofluorescence laser confocal microscopy (17). Twenty-microgram aliquots of total membranes isolated from oocytes injected with the indicated mRNA were subjected to immunoblot analysis using polyclonal antibodies specific for either Glut1 or Glut2. The Glut2 isoform-specific antiserum was raised against a synthetic peptide corresponding to the COOH-terminal 14 residues of human Glut2 (East Acres Biologicals, Southbridge, MA) and was used at 1:1000 dilution for immunoblots and at 1:50 dilution for immunocytochemistry. The Glut1 isoform-specific antiserum F-350 (20) was raised against a synthetic peptide corresponding to the COOH-terminal 15 residues of human Glut1 (13). IgG was purified from F-350 using a protein A column. The IgG fraction was used at 10 μg/ml for immunoblots and at 20 μg/ml for immunocytochemistry. Twenty micrograms of total human liver protein were loaded on the Glut2 gel as a positive control and size standard. Twenty nanograms of purified human erythrocyte glucose transporter were loaded on the Glut1 gel as a positive control and size standard.

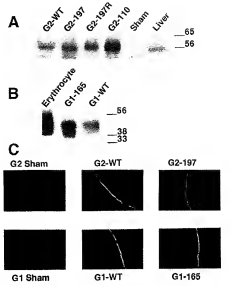

FIG. 2. **Expression of wild-type and mutant glucose transporter proteins in *Xenopus* oocytes.** Oocytes were injected with wild-type or mutant glucose transporter mRNAs. Three days after injection, oocytes either were used for membrane preparation or were frozen, sectioned, and subjected to indirect immunofluorescence laser confocal microscopy. *A*, immunoblot of membranes isolated from oocytes expressing wild-type Glut2 (*G2-WT*) or Glut2 mutants (*G2-197*, *G2-110*). *G2-197R* represents a Glut2 clone in which the valine 197 → isoleucine mutation was reverted back to valine. *Sham* represents membranes from water-injected oocytes. *B*, immunoblot of membranes isolated from oocytes expressing wild-type Glut1 (*G1-WT*) or the Glut1-165 mutant (*G1-165*). *C*, confocal immunofluorescence micrographs of oocytes expressing glucose transporter mutants. The sham-labeled pictures illustrate the level of background staining in sections of water-injected oocytes that were treated identically to sections from the corresponding mRNA-injected oocytes. The abbreviations are defined in the legend to Fig. 1.

RESULTS AND DISCUSSION

Injection of wild-type Glut2 mRNA into oocytes resulted in a 58-fold increase in the uptake of [^{3}H]2-deoxyglucose relative to uptake in water-injected control oocytes (Fig. 1). The isoleucine 110 substitution did not appreciably affect transport activity. However, substitution of isoleucine for valine 197 dramatically reduced transport activity. To determine whether mutant Glut2 protein was synthesized in the oocyte, immunoblots were conducted on oocyte membranes using antiserum raised against a synthetic peptide corresponding to the carboxyl terminus of human Glut2 (Fig. 2*A*). This antibody was also used to localize the Glut2 proteins by indirect immunofluorescence laser confocal microscopy in frozen oocyte sections (Fig. 2*C*). Both mutant proteins were produced in oocytes at somewhat greater levels than the wild-type transporter, and the proteins exhibited very similar subcellular distributions with pronounced plasma membrane staining.

The effect of the isoleucine 197 change on transport activity was most surprising, since this mutation substitutes one hydrophobic transmembrane residue for another, and isoleucine and valine differ in structure by only a single methylene group. The substitution of isoleucine for valine is one of the more common amino acid changes observed at the equivalent positions of homologous proteins (21). To be certain that the transport defect was due to the desired mutation rather than an undetected spurious mutation, isoleucine 197 in the mutant cDNA clone was mutated back to valine. The back-mutated Glut2 protein was fully functional when expressed in oocytes (Fig. 1). The equivalent mutation was also introduced into human Glut1, changing valine 165 to an isoleucine residue. This Glut1 mutant also exhibited a severe reduction in transport activity (Fig. 1). However, the mutant Glut1 protein was synthesized in the oocytes at high levels and exhibited the same subcellular distribution as wild-type Glut1 (Fig. 2, *B* and *C*).

These data indicate that the valine residue at position 197 of Glut2 and the equivalent position 165 of Glut1 lie in a particularly crucial region of the protein with respect to transporter function. Interestingly, this residue is approximately one helical turn distant from glutamine 161 of Glut1, a residue that we have recently demonstrated affects the binding of the exofacial ligand, ethylidene glucose.[2] This suggests that the valine side chain lies very near to the exofacial substrate binding site within a putative aqueous tunnel formed by the clustering of transmembrane helices (22). The presence of a bulkier side chain at this critical site may be sufficient to block substrate binding or passage of substrate through the aqueous chamber formed by the transporter transmembrane helices.

The presence of the dysfunctional isoleucine 197 mutation in at least one diabetic patient is intriguing, since mutations in the β cell Glut2 transporter were predicted to be causally involved in the pathogenesis of non-insulin-dependent diabetes mellitus (6, 7). Unfortunately, it is not possible to determine whether the isoleucine 197 mutation is causally involved in the diabetes of the single patient identified, because relatives were not available for linkage analysis. The mutation within codon 197 of Glut2 appears to be rare. No mutant alleles were found among 36 additional female African-American NIDDM pa-

[2] M. Mueckler, W. Weng, and M. Kruse, submitted for publication.

tients that were screened for the present study. Thus, the mutation has been detected in one of 84 overt diabetic African-American women. The mutation was not detected in any of 75 Caucasian NIDDM patients that were screened for this study.

Recent biochemical evidence presented by Hughes *et al.* (23) suggests that Glut2 may play an active role in glucose-stimulated insulin secretion. Their data suggest that the role of Glut2 in glucose-stimulated insulin release is not related to the overall rate of glucose flux and metabolism, but rather may involve physical coupling of Glut2 with cellular proteins involved in glucose signaling. Because the diabetic patient possessing the isoleucine 197 mutation has only one normal Glut2 allele, a possible scenario is that the expression of Glut2 protein has been reduced by ~50% in the β cell, liver, kidney, and intestine. A reduction in β cell Glut2 protein might reduce glucose-stimulated insulin release and thus contribute to the development of diabetes in this patient. This would be consistent with the known role of heterozygous glucokinase gene mutations in the development of maturity onset diabetes of the young (24). However, we cannot rule out the possibility that there is an as yet undetected mutation present in the other Glut2 allele of this patient. Further work is necessary to definitively conclude whether single-allele disruptions in the Glut2 gene are involved in the pathogenesis of diabetes.

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